THE EFFECTS OF INORGANIC SALTS ON DIVIDING CELLS.

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(Plate viii; two Text-figures.)

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Synopsis.

Onion root tips treated with certain inorganic salts showed abnormal cell division and a reduction in the number of dividing cells with time. The addition of a low concentration of other salts to the medium neutralized the effect of the single inorganic salt. This inhibition of the cell division process seems to be due to ion unbalance in the external solution, rather than the specific effect of any inorganic ion.

Introduction.

A wide variety of organic substances is known to produce abnormalities in mitosis in both plant and animal cells, and the effects of some inorganic salts on the cell division process have also been described. In 1945 Levan, who treated onion root tips with a number of inorganic salts, mostly nitrates, found that solutions of the salts of 27 different metals produced cytological changes in the cells of the meristem. Galinsky (1949) reported the inhibition of cell division in the same material, by solutions of Na_2HPO_4, $NaHPO_4$, and KH_2PO_4. He attributed the observed abnormalities to the presence of the phosphate and compared them to the effects produced by colchicine and acenaphthene. Using chick cells in tissue culture, Hughes (1952) found that abnormal tonicity upset cell division by suppressing spindle formation and producing blocked metaphase. This raises the possibility that cytological abnormalities in the presence of inorganic salts may be due to an ion unbalance in the solution, rather than to a specific effect of any inorganic ion. When a phosphate buffer was used to control the pH in some experiments with onion seedling roots (Hindmarsh, 1953), it was noticed that abnormal mitoses were produced in the root meristems, and experiments were designed to find whether this was caused by the phosphate or by the univalent ions in the solution.

Materials and Methods.

Onion seeds were surface sterilized in a saturated solution of bleaching powder for 5 to 10 minutes, set out in sterilized petri dishes on filter paper moistened with culture solution and incubated for 7 days at 22°C. The culture solution was KNO_3 0·0025M, $Ca(NO_3)_2$ 0·0025M, $MgSO_4$ 0·001M, and KH2PO4 0·0005M (Hoagland and Broyer, 1936) at a pH of 5·3.

Preliminary examinations of the relationship between root length and the number of dividing cells in the root tips were made to find the least variable material for cytological investigation. Counts of dividing cells showed that seedlings with roots from 6·0 to 11·0 mm. after 6 to 7 days proved less variable than shorter or longer roots. The over-all mean for four experiments was 290·5 for 74 roots, and the pooled standard deviation within these four groups (calculated from the mean of each group) was 67·7.

Roots were treated by floating seedlings on 20 c.c. of the test solution in 150 c.c. Erlenmeyer flasks. All the solutions were autoclaved without any appreciable change in pH. The few seedlings which sank during the course of an experiment were discarded.

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Root tips were fixed in acetic alcohol and stained in 1·0% aceto-orcein. To count the number of dividing cells in each stage of division, roots were heated in acidified aceto-orcein stain and the meristematic region stained more deeply than the remainder of the root. This deeply stained tip was removed just behind the meristematic zone, and cut into 4 pieces in fresh 1·0% stain. These pieces were squashed into a single cell layer by pressure on the coverslip and division stages scored during a systematic examination of the whole slide.

The buffer solution was a mixture of 2 parts $Na_2HPO_4.12H_2O$ (23·9g/l), and 8 parts KH_2PO_4 (9·07g/l) with a final concentration of 0·06M and a pH of 6·2. Roots were treated with 0·06M KCl, 0·06M, 0·03M, and 0·015M phosphate, and with each solution combined with Hoagland's culture solution. Controls were grown in the culture solution.

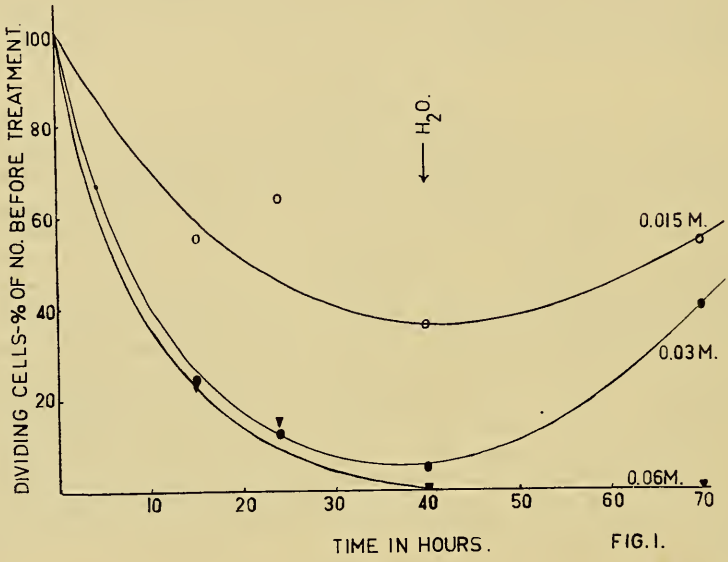

FIG. 1.

Results.

a. The effect of the buffer solution. There was no evidence of any chromosome fragmentation with this treatment, but other cytological abnormalities were produced. The number of dividing cells in root tips was reduced by all concentrations indicating

Table 1.

Time in Hours.	Phosphate Concentration.		
	0·015M.	0·03M.	0·06M.
15	Numerous normal dividing cells.	Fewer divisions than normal. Chromosomes short and thick, otherwise normal.	Blocked metaphase. Few anaphase—all irregular.
24	Mainly normal. Few blocked metaphase.	Few divisions, some normal, some blocked.	Very few divisions, all abnormal. No anaphase and telophase.
40	Mainly normal. Some cells have short, thick chromosomes. Few blocked metaphase.	Most cells resting nuclei, but some roots have few prophase and blocked metaphase.	All roots have only interphase nuclei.
40+30 in water	All normal dividing cells.	All normal dividing cells.	All interphase nuclei.

that the onset of the cell division process was prevented (Text-fig. 1). The spindle mechanism was also affected and cytological abnormalities varied with the concentration of the phosphate buffer and time (Table 1).

The first observed effects were shortening, thickening of chromosomes in otherwise normal metaphase and anaphase stages, followed by irregular metaphase and anaphase stages with some chromosomes left off the metaphase plate or lagging at anaphase. Finally all early stages were affected as they were unable to pass metaphase; blocked metaphase occurred instead of normal metaphase plate formation (Pl. viii, A and B). Some binucleate cells formed, but these were rare, suggesting that there is little effect on cells which were in anaphase and telophase at the time of treatment.

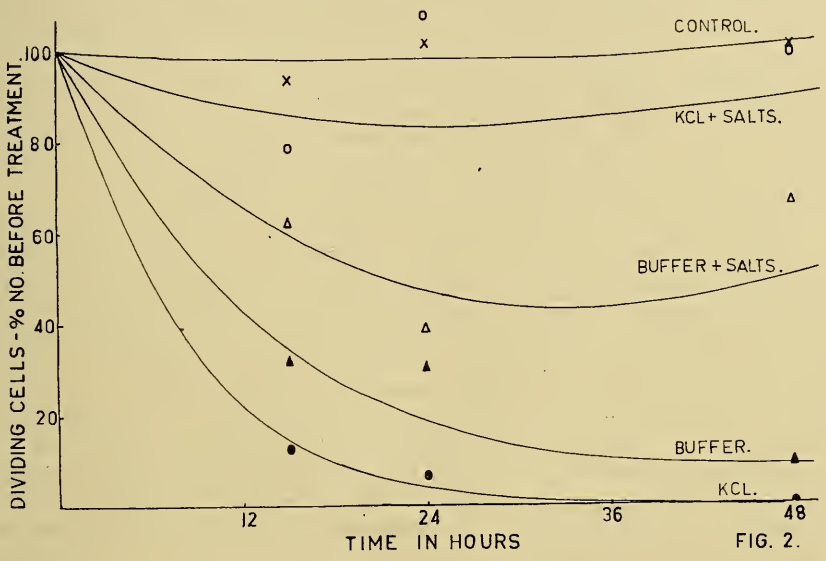

FIG. 2.

The cells of roots returned to water or culture solution after treatment with 0·06M phosphate buffer for 40 hours did not begin division even after 30 hours. Most cells appeared to be quite healthy, but some were pycnotic. The roots remained white and turgid and were apparently still alive. There was no "stickiness" of chromosomes such as was observed during treatment with nitrophenols (Levan and Tjio, 1948; Hindmarsh, 1951). After shorter treatment at 0·06M and after treatment with lower concentrations the roots recovered in water or mineral solution and commenced normal division.

In roots treated with 0·06M phosphate buffer and mineral solution together, no cytological abnormalities were observed. All stages of normal mitosis were found in these roots, but there was a gradual reduction in the number of dividing cells with time (Pl. viii, F). This reduction was not as great as with phosphate buffer alone (Text-fig. 2).

b. The effect of a single salt solution. The entry of cells into cell division was affected more by 0·06M KCl than by the same concentration of phosphate buffer (Text-fig. 2). KCl solution also produced extra contraction of the chromosomes in metaphase and anaphase stages, and a few blocked metaphase stages at 15 hours (Pl. viii, C, D and E). The addition of a culture solution to the KCl solution reduced the effect on the number of dividing cells (Text-fig. 2), and in roots so treated the chromosomes as well as the division process were perfectly normal (Pl. viii, G).

Discussion.

The results of these experiments show that the ionic composition of external solution can influence the course of the cell division process.

Chemical inhibition of the mitotic cycle can occur in one of three ways. Some chemicals inhibit or destroy the spindle mechanism, leading to the formation of blocked metaphase, abnormal anaphase and telophase in the early stages of treatment, and ultimately to the complete absence of anaphase and telophase, and to polyploidy, e.g., colchicine. A second group has a similar effect, but also prevents the onset of prophase leading to a reduction in the number of dividing cells with time, and as each cell goes through only one cycle during treatment there is no polyploidy, e.g., sulphanilamide. A third group causes chromosome breakage, e.g., nitrogen mustards.

The cytological abnormalities produced in these experiments are similar to those of the second group of chemicals. A sodium-potassium phosphate buffer produced spindle abnormalities in meristematic cells, only in the absence of other salts. Both the phosphate buffer alone, and the buffer with mineral salts added, reduce the number of dividing cells by preventing the entry into prophase, but this effect is greater with the buffer solution only. The spindle mechanism was inhibited by the buffer alone, but not by the combined solution. Similar effects were obtained with KCl solution, which has less effect on the spindle mechanism and more effect on the interphase to prophase stage of the cycle. Again, the addition of a culture solution with a low concentration of divalent as well as monovalent ions allowed the division process to continue in a normal manner and reduced the effect on the number of dividing cells.

An analogous phenomenon is known in root growth in culture solutions. It is generally agreed that there must be a suitable balance of the various ions in a solution, to maintain normal plant growth. The toxic action of a single salt such as KCl is reduced by the addition of quite low concentrations of a divalent salt. Antagonism, the influence of one ion in preventing the toxic symptoms due to another ion alone, is well known, but imperfectly understood. The best known example in plants, the antagonism of Ca or K by the other ion, presumably occurs because the presence of K or Ca alone in solution results in structural disorganization of the cytoplasm. In these experiments the ion unbalance in the external solution affects the cell division process, as the cytological abnormalities produced by both the phosphate buffer and the KCl were neutralized at least in part by the addition of low concentrations of other salts to the solution. This suggests that the cytological abnormalities may be the indirect result of physiological changes in the cells, and are not due to the specific effect of any inorganic ion. This inhibition of the cell division process may be one factor which contributes to the inhibition of root growth in an unbalanced solution.

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* Seen in abstract only.

EXPLANATION OF PLATE VIII.

A-B: Blocked metaphase in sodium-potassium phosphate buffer. C-E: Abnormal metaphase and anaphase in KCl. F: Normal cell division in phosphate buffer + culture solution. G: Normal cell division in KCl + culture solution.